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The tyrosine rich eggshell protein referred to as F4 has been modelled exhaustively both by computer simulation and by physical studies of synthetic peptides. Our conclusion is that the tyrosine rich repetetive region of this protein probably forms a left handed alpha helix. I have published the suggestion, based on the established protein sequences, that the absence of phenylalanine residues may suggest a role in electron transport.

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Objectives. The objective of this project was to further elucidate the mechanisms by which the schistosome eggshell becomes cross-linked. During the course of this project our aims were modified considerably in the light of our data. The most important change in my thinking was to accept the possibility, I might even say probability today, that there is no conventional phenol oxidase enzyme responsible for the final steps in the cross-linking process. This point of view has been argued in some detail in the publications listed and attached.

Accomplishments: The repetitive tyrosine rich protein which we refer to as F4, has been the major focus of our work. We have continued to refine our analysis of this unusual proteins possible secondary structure and these results have been published in detail (Middaugh et al., 1993). Our data continues to support the unusual conclusion that this protein, comprising only L-amino acids adopts a left-handed alpha helical conformation.

I have further suggested (Cordingley et al., 1993) based upon sequence data from two laboratories that the F4 protein may act as an "electron transport chain" during eggshell cross-linking. Our attempts to design experiments to test this hypothesis have been unsuccessful. The readiness with which the cross-linking reactions occur is in itself a major stumbling block to characterizing the system and we have been unsuccessful in

isolating stable intermediates of the cross-linking process.

We have begun making peptides with specific substitutions to try to create peptides in which we can measure electron transfer (or perhaps more simply "energy transfer") along the peptide. It has proved possible in other proteins to use tryptophan residues to introduce "energy" into proteins by irradiating with UV light of specific wavelengths and to detect energy transfer to other distant groups within the protein. To try to exploit this we have replaced one tyrosine residue with a tryptophan and another tyrosine residue, more C-terminal in the peptide with a cysteine residue. The cysteine residue allows us to couple reporter groups or we may be able to detect direct reduction of a disulfide bond at this position. Preliminary observations show that the peptides with the tryptophan and cysteine substitutions retain the left handed structure found in the native peptide.

The publication of another complete sequence of an F4 homologue reinforces the points made previously regarding the absence of Phe and Trp substitutions for the tyrosines in F4. There is one Phenylalanine, but it is in the signal peptide sequence. There are 120 tyrosine residues in the remainder of F4 and not a single Phenylalanine or tryptophan. The sheer number of tyrosine residues renders the lack of Trp or Phe substitutions much more significant than in a protein with only a few tyrosine residues. Clearly there must be very strong selective pressure preventing acceptance of these substitutions.

The attached reprints spell out our thinking on eggshell cross-linking in schistosomes and on the points outlined here and I see no particular advantage to repeating these ideas here. The interested reader is referred to the attached reprint collection.

Publications.

Cordingley, J.S., Thomson, J.A., and Middaugh, C.R. (1993). Is the Tyrosine Rich Eggshell Protein of Schistosoma mansoni and electron transport chain? Mat. Res. Soc. Symp. Proc. *292*, 69-76.

Middaugh, C.R., Thomson, J.A., Burke, C.J., Mach, H., Naylor, A.M., Bogusky, M.J., Ryan, J.A., Pitzenberger, S.M., Ji, H., and Cordingley, J.S. (1993). Structure of synthetic peptides derived from an eggshell protein of Schistosoma mansoni. Protein Sci. *2*, 900-914.

Wells, K. and Cordingley, J.S. (1991). Detecting proteins containing 3,4-dihydroxyphenylalanine by silver staining of polyacrylamide gels. Anal. Biochem. *194*, 237-242.

Wells, K.E. and Cordingley, J.S. (1991). *Schistosoma mansoni*: Eggshell formation is regulated by pH and calcium. Exp. Parasitol. *73*, 295-310.

Wells, K.E. and Cordingley, J.S. (1992). The Cell and Molecular Biology of Eggshell Formation in Schistosoma mansoni. In Structure, Cellular Synthesis and Assembly of Biopolymers. S.T. Case, ed. (New York: Springer-Verlag), pp. 97-113.